Chemically-optimized stereopure oligonucleotides direct ADAR-mediated RNA editing of SERPINA1 transcripts, yielding functional α1-antitrypsin protein in a mouse model for α1-antitrypsin deficiency

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Forward-looking statements

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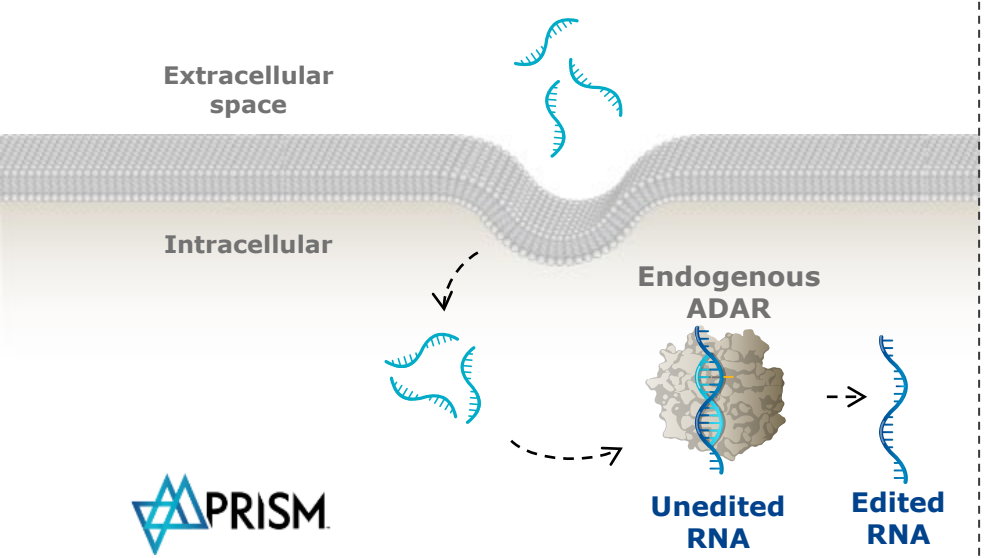
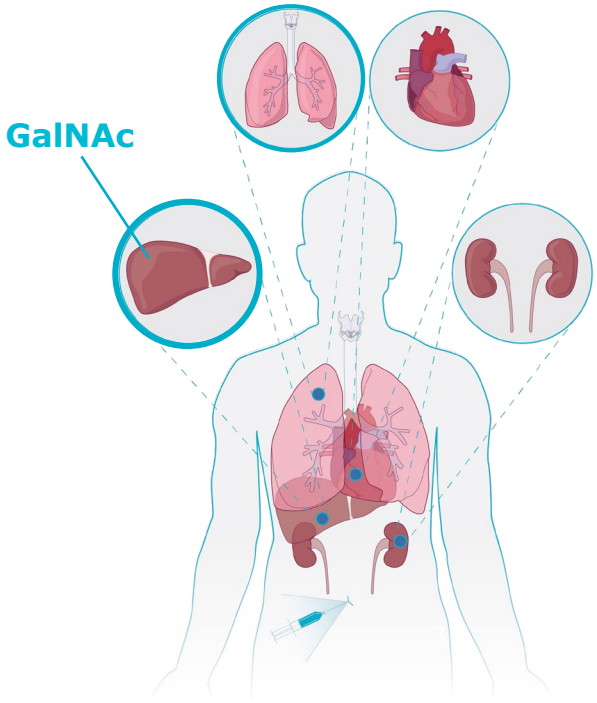
PRISM enables practical approach to RNA editing without need for viruses or exogenous protein

Wave ADAR-editing oligonucleotides



PRISM

- ✓No delivery vehicle required
- ✓No exogenous proteins necessary
- ✓Potential for reduced off-target effects



Subcutaneous administration

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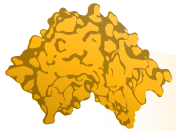
An ADAR editing approach to correct alpha-1 antitrypsin deficiency

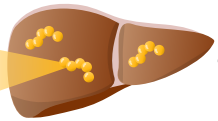
Alpha-1 antitrypsin deficiency (AATD)

Most common cause is mutation in *SERPINA1* Z allele (PI*ZZ)

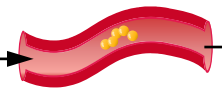
~200K people in US and EU with ZZ genotype



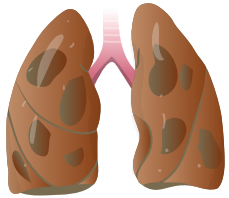
Z-AAT
misfolded protein prone to aggregation



Inability to secrete polymerized Z-AAT
liver damage/cirrhosis



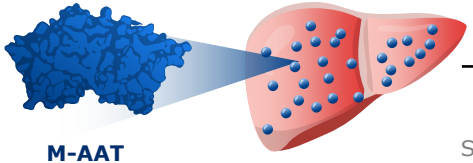
☒ No M-AAT



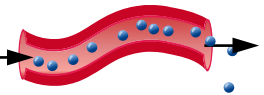
Open to unchecked proteases
inflammation / lung damage

Wave's ADAR editing approach

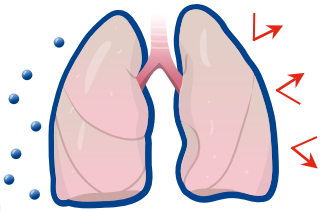
Correct Z allele mRNA to healthy M allele



M-AAT
Wild-type AAT protein



Secretion into bloodstream



Lungs protected from proteases

- ✓ Restores M-AAT physiological regulation in liver
- ✓ Reducing Z-AAT protein aggregation
- ✓ Restores circulating, lung-bound M-AAT

Risk of disease

Null *(no AAT)*	**Highest risk** *(lung)*
PI*ZZ	**High** *(lung + liver)*
PI*SZ	
PI*MZ	**Low**
PI*MM	**Normal**

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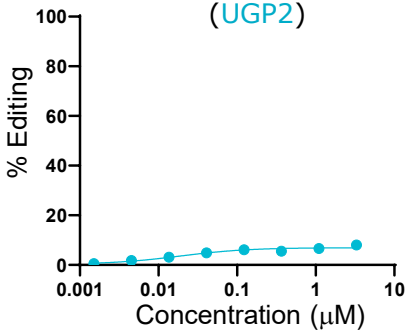
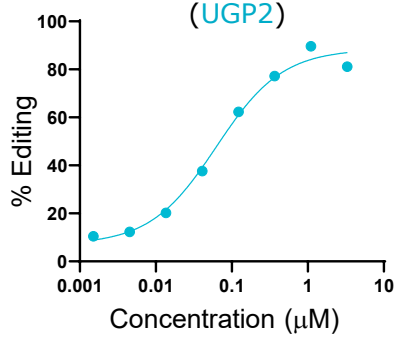
Fregonese, Respir Med, 2008, Strnad, NEJM, 2020

Species differences in ADAR activity necessitate transgenic human ADAR mouse model

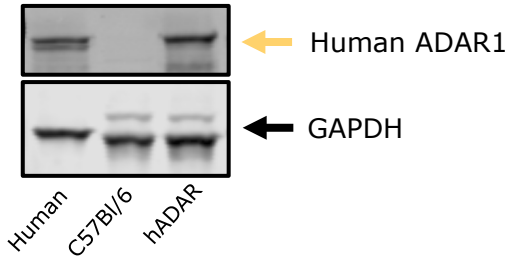
Mouse hepatocytes
(UGP2)



huADAR mouse hepatocytes
(UGP2)



ADAR expression in hepatocytes



huADAR mouse

Genotype	
✓	huADAR
	Human ADAR expressed in all tissues

- Transgenic mouse expressing **human ADAR1**
- Expression of ADAR approximates expression in human liver

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Focused on restoring wild-type M-AAT *in vivo*

NSG-PiZ

Genotype
✓ huSERPINA1-Pi*Z
Human Z-AAT protein expressed in liver

huADAR mouse

Genotype
✓ huADAR
Human ADAR1 expressed in all tissues

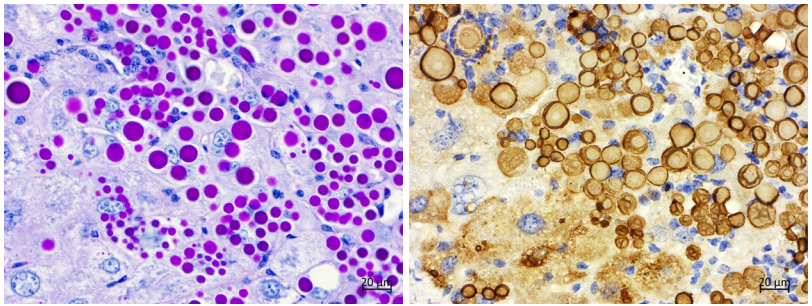
huADAR/AATD mouse

Genotype	Pathology
✓ huADAR	Liver pathology, Z-AAT protein in serum and liver
✓ huSERPINA1-Pi*Z	

AATD mouse

Genotype	Pathology
✗ huADAR	Liver pathology, Z-AAT protein in serum and liver
✓ huSERPINA1-Pi*Z	

huADAR/AATD mouse



- Liver AAT aggregation observed in AATD is recapitulated in mouse model

AATD: Alpha-1 antitrypsin deficiency, Z-AAT: mutated protein, M-AAT: wild-type human AAT protein
(Left) Hematoxylin and PAS-D stain and (Right) immunohistochemistry for AAT protein with hematoxylin counterstain in the huADAR/AATD mouse liver

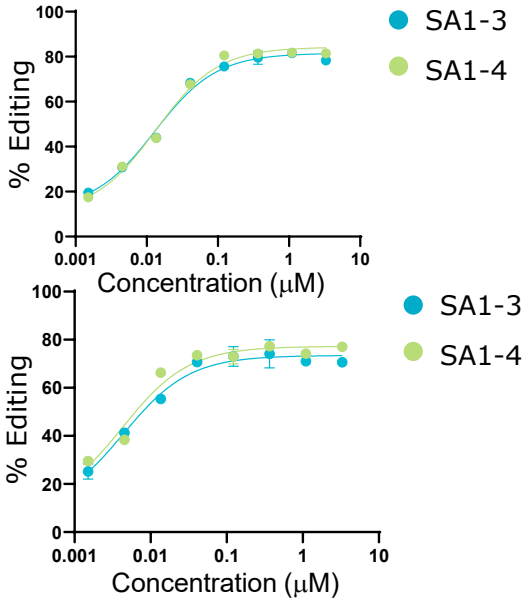
Achieving 40% editing of Z allele mRNA at single time point

SERPINA1 Z allele mRNA editing levels nearing correction to heterozygote (MZ)

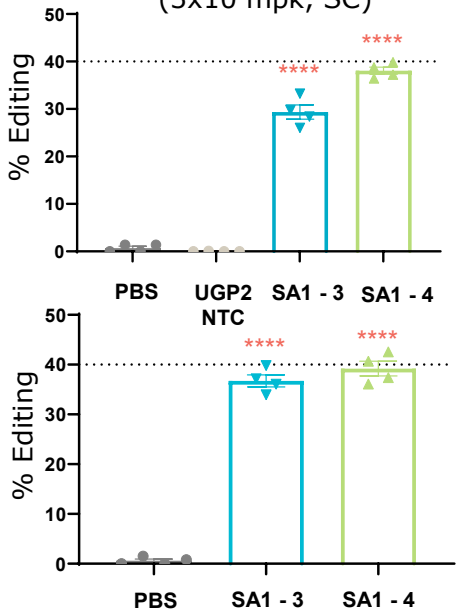
huADAR/AATD mice

AATD mice

SERPINA1 editing, *in vitro*
(Primary hepatocytes)



SERPINA1 editing, mouse
(3x10 mpk, SC)



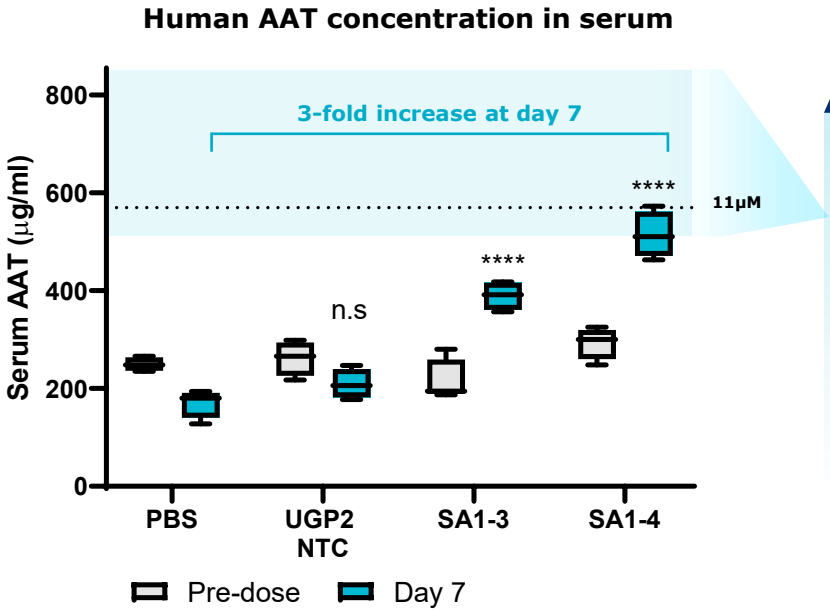
- GalNAc-conjugated compounds
- Up to 40% editing of Z allele mRNA in liver of transgenic human ADAR1 mice at day 7
- Comparable editing in mice lacking human ADAR (AATD mice)
- Highly specific editing (no bystander edits)

Z allele mRNA editing *in vivo* | **AAT protein increase** | **Wild-type M-AAT functional**

HuADAR/AATD or AATD mice administered PBS or 10 mg/kg oligo on days 0, 2, and 4. Samples collected on day 7. Stats: One-way ANOVA **** P<0.0001; NTC: non-targeting control

Achieving therapeutically meaningful increases in circulating human AAT protein

3-fold increase in circulating human AAT as compared to PBS at initial timepoint



AAT serum levels by genotype

PI*MZ	~3 to 5-fold increase
PI*SZ	~2-fold increase
PI*ZZ	~3 – 7 uM

✓ Z allele mRNA editing *in vivo*

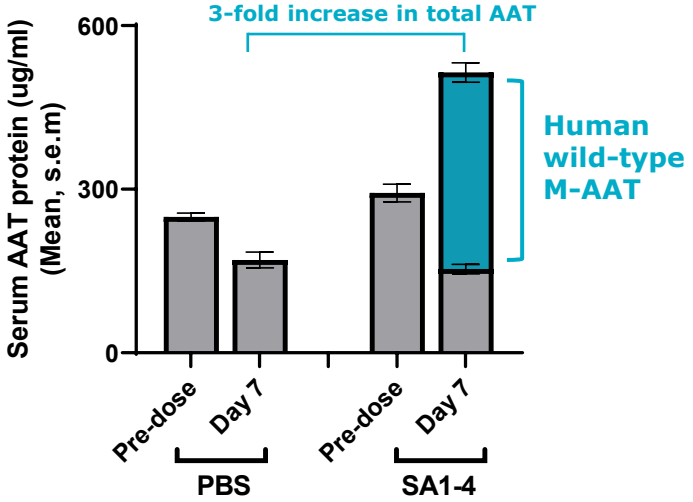
✓ AAT protein increase

Wild-type M-AAT functional

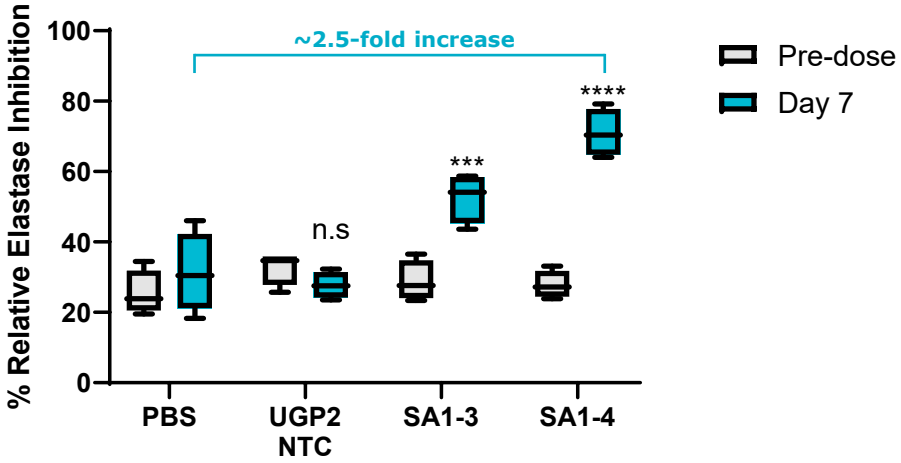
Statistics (ELISA): Matched 2-way ANOVA with correction for multiple comparisons (Bonferroni) was used to test for differences in AAT abundance in treated samples compared to PBS ns nonsignificant, ****P<0.0001; de Serres et al., J Intern Med. 2014; NTC: non-targeting control

ADAR editing restores circulating, functional M-AAT

Wild-type M-AAT detected with ADAR editing



Significant increase in neutrophil elastase inhibition with ADAR editing



✓ Z allele mRNA editing *in vivo*

✓ AAT protein increase

✓ Wild-type M-AAT functional

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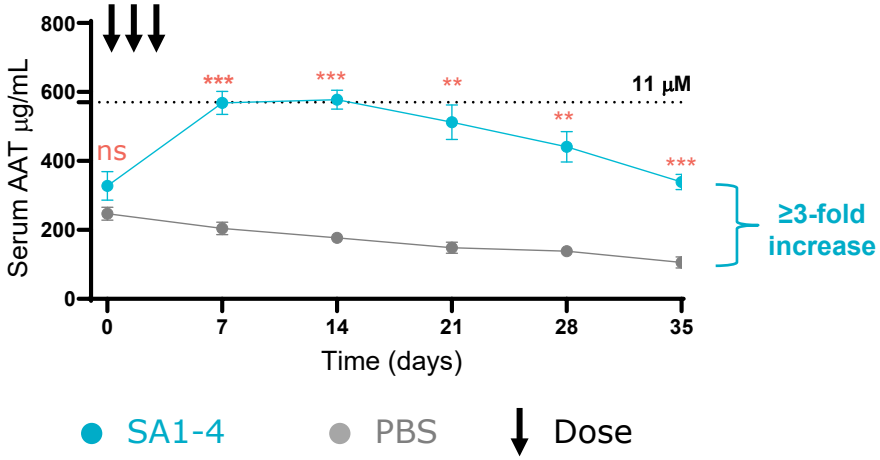
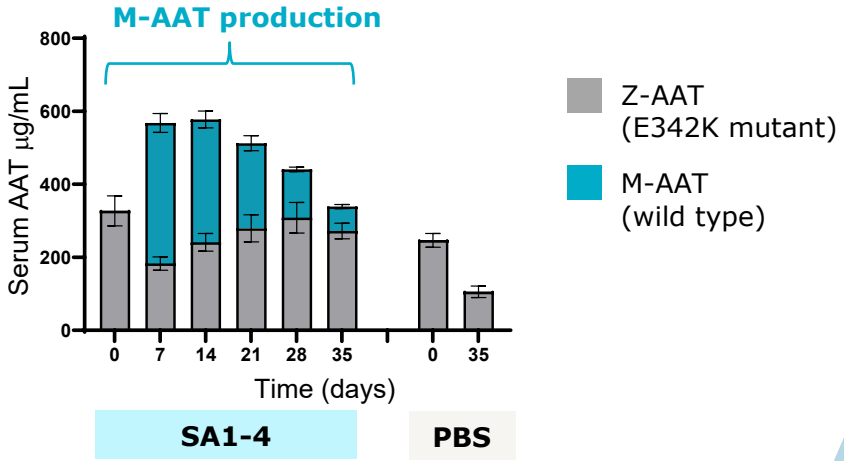
Left: Mass spectrometry and ELISA Right: (Elastase inhibition): Matched 2-way ANOVA with correction for multiple comparisons (Bonferroni) was used to test for differences in elastase inhibition activity in serum collected at day 7 vs pre-dose for each treatment group, ns nonsignificant, *** P<0.001, **** P<0.0001; NTC: non-targeting control

Durable increase in serum AAT in mouse model

Serum AAT with loading dose
(3x10 mpk, SC)



Serum AAT
(3x10 mpk, SC)



Durable mRNA editing in vivo

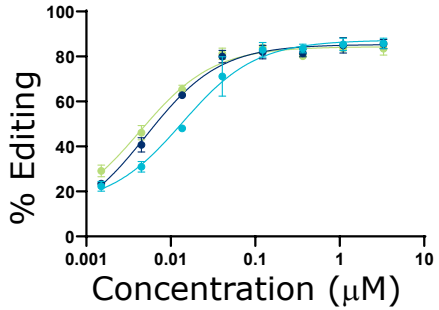
Increase in M-AAT in serum

(Left) Humanized ADAR mice administered 3x10 mpk doses on days 0, 2 and 4. Serum was collected on days 7, 14, 21, 28, and 35. AAT levels quantified by ELISA. Data are presented as mean ± sem. Stats: Matched 2-way ANOVA ns nonsignificant, ** P<0.01, *** P<0.001. (Right) Proportion of AAT in serum that is E type (mutant) or M type (wild type) as measured by mass spectrometry.

Optimization further improves mean editing

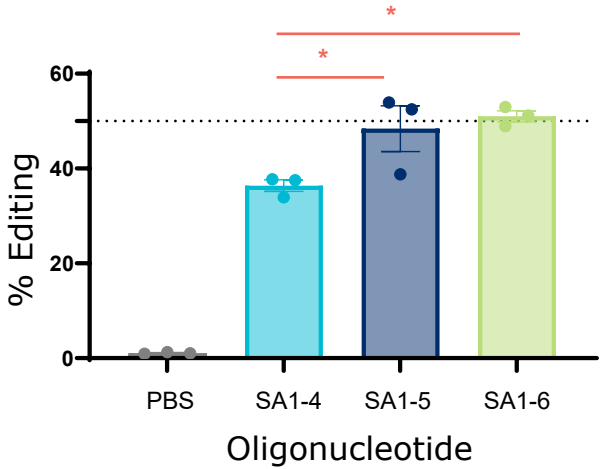
50% mean editing observed with 50% lower dose in mice

SERPINA1 RNA editing *in vitro*



	SA1-4	SA1-5	SA1-6
EC_{50}(nM)	13.7	5.1	4.3

SERPINA1 RNA editing huADAR mouse
(3x5 mpk, SC)



(Left) Primary mouse hepatocytes were treated with increasing doses of various GalNAc conjugated oligonucleotides to generate dose-response curves. EC_{50}s were calculated from the curves. (Right) The same oligonucleotides were administered to mice (3x5 mpk) on days 0, 2 and 4. Livers were collected on day 7, and SERPINA1 editing was quantified by Sanger sequencing (shown as mean ±. sem) Stats: One-way ANOVA was used to test for differences in editing between SA1-4 and other oligos * P<0.05

Summary

- Up to 50% editing of *SERPINA1 Z* allele mRNA in liver, nearing correction to heterozygotes (MZ)
- Z allele mRNA editing results in therapeutically meaningful increase in circulating functional wild-type M-AAT protein *in vivo*
- Restoration of wild-type M-AAT is durable
- Production of M-AAT suggests clearance of Z-AAT from liver
- Ongoing optimization efforts continue to improve editing efficiency and duration of activity, enabling comparable outcomes with lower doses

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